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SOME NEW METHODS IN NEMATODE TECHNIQUE*

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The following article is a digest of some methods for the handling of nematodes, which the author has worked out and found to be rapid and reliable, eliminating considerably the difficulties encountered in dealing with these most refractory organisms.

The material freshly collected and freed from adhering dirt and slime is placed in the following killing, clearing and dehydrating fluid, for convenience designated as Carnoy-phenol:

Absolute alcohol	20 c.c.
Chloroform	15 c.c.
Glacial acetic acid	5 c.c.
Phenol crystals to raise the volume by	10 c.c.
Total	50 c.c.

As this mixture is water free and very corrosive, it must be stored in glass stoppered bottles until used and also should not be kept more than 2 weeks as an ester formation decreases its efficiency. In preparing specimens, it is most easily handled in Syracuse crystals, the edges of which have been dipped in hot paraffin to prevent the liquid from creeping over the rims and furthermore operations in these dishes may be easily controlled under a binocular or a compound microscope.

If material is killed in Carnoy-phenol, only 2 operations are required to bring the objects into paraffin or balsam, and one to clear them in glycerine; if killed in other media and stored in alcohol, 3 operations will attain the same end. Nematodes may be taken from 70 to 80 per cent. alcohol, glycerine, lacto-phenol, or formol in which they have been stored and placed directly in the fluid. Smaller worms are cleared almost instantly so that a rapid survey if desired may be made of their internal organization after which they may be removed to 95 or 80 per cent. alcohol for storage. If the larger specimens do not clear sufficiently at first, almost any degree of clearing may subsequently be obtained by allowing the fluid in which the worms are placed to evaporate, the degree of clearing being proportional to the amount of evaporation. Still greater transparency is obtained by adding glacial acetic acid and phenol to the worms.

With specimens now in the fluid they may be prepared either for sectioning or for mounting in balsam with equal ease. Oils of synthetic wintergreen and oleum cidri ligni (Merk) or chloroform may be dropped *slowly* into the dish with the specimens and mixed thoroughly by agitation. The change must be very gradual at first, when the tissues are hardening but as soon as the mixture is three quarters clearing fluid, the greater part may be drawn off and the pure liquid added more rapidly and allowed to remain for 10 to 15 minutes or longer. If now infiltration by paraffin is desired the wax is shaved into the dish with the specimens in clearing fluid and the whole set aside in a slightly warm place for 2 hours when the worms may be placed in pure paraffin, melting at 58 C. and after proper infiltration imbedded. Most stains follow Carnoy-phenol admirably.

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* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 209.

To mount whole preparations in balsam the procedure is the same, including the bringing of the worms into clearing fluid; wintergreen is here to be preferred to the other clearing fluids in general laboratory use because of its rapid penetrating power; xylol shrinks tissues too readily and should be entirely avoided. Now the Syracuse crystal bearing the worms in a small quantity of oil is tipped only slightly and a large drop of pure, unthinned, paper-filtered Canada balsam, is placed on the sloping bottom of the dish away from the worms and the whole covered. The resin will flow slowly down and diffuse throughout the oil and specimens in the course of 2 or 3 hours. Should the resulting resinous mixture be too thin to dry rapidly upon mounting the objects, more balsam may be added as before. It is important not to rush this process because the thinner medium within the worms will move through to the exterior faster than the balsam can penetrate to the interior with the result that the pressure becomes less within than without and unless the cuticula is thick, collapsing will result; but in all cases the more volatile fluids will vaporize under this reduced pressure and fill the body cavity and interstices between the organs with gas so that the preparations are again valueless, being utterly opaque. If collapsing has not taken place, the difficulty may be remedied by thinning the balsam with chloroform or benzol until the bubbles are gone, then controlling evaporation until the thickness of the fluid is again suitable for mounting. However, should collapsing as well have occurred, and should the specimens be valuable enough to warrant saving, restoration may be accomplished by running the worms back to Carnoy-phenol and leaving them there until the collapsed portions have plumped out. If this does not occur spontaneously, a slight manipulation by rolling the worm gently will usually restore shape. Where the specimens are large, it is desirable to puncture the cuticula in several places before balsam clearing, taking care not to injure the underlying organs.

Except for low power work with a microscope, toto staining is of little value in examination of relatively large specimens because of the marked tendency it has to mask the finer details of structure which one is desirous of seeing when using higher magnifications. Much greater latitude for observation is better obtained by utilizing degrees of clearing rather than staining. Permanent mounts may be made of glycerine-prepared specimens in glycerine jelly properly sealed against evaporation, or material may be mounted after suitable preparation either in camsal-balsam, cedar immersion oil, or Canada balsam. These 4 mounting media will give a differential clearing indicated by the following approximate indices of refraction: 1.476, 1.47, 1.520, and 1.535 (Lee 1913), respectively. To prepare the specimens for passage into these media, they are first placed in Carnoy-phenol and then brought into the clearing fluids most suitable for passage into the mounting medium. For glycerine jelly mounts, the phenol reagent is replaced by pure glycerine; camsal-balsam is preceded by clearing the material in camsal, a liquid formed by the mutual solution of salol (phenyl-salicylate) and gum camphor; immersion oil follows thin cedar oil; and Canada balsam replaces oil of wintergreen. The process of clearing is accomplished as previously explained.

Another excellent medium for small, very transparent worms, is "Diaphane" a resinous medium employing gum Sandarac on the order of Gilson's "Euparal" which, because of its low index of refraction shows greater detail in the cleared specimens than balsam. The nematodes are cleared carefully from the phenol reagent by camsal and allowed to harden for a short time in this fluid. Then they may be transferred to diaphane, diluted to about one half strength by absolute iso-butyl alcohol, and allowed to clear by gentle evaporation of the alcohol. A slightly greater degree of clearing may be obtained by preparing the worms in the same manner and transferring to pure Canada balsam with 5 to 10 per cent. camsal well diluted with iso-butyl alcohol. This, too, makes an admirable, but very slow drying, tough, elastic medium.

It sometimes happens that specimens will accidentally become dried out through breakage of the glass container or neglect to stopper it properly. Such objects may be very well and often completely restored by the following method, such that staining, sectioning or toto-mounting may be undertaken. The dried worms are soaked in 35 to 50 per cent. alcohol to which an equal volume of lactic acid is added. If they do not soften and plump out to normal size, more acid may be added. When restoration is complete, the nematodes must be freed of the acid by thorough rinsing in 70 per cent. alcohol when they are ready for further operations. This method has been applied to cestodes, trematodes, arthropods, and their larvae with equally remarkable results.

LITERATURE CITED

Enzyklopädie der Mikroskopischen Technik. 1910. I. 800 pp.

Gilson, G. 1909.—Un nouveau médium solidifiable pour le montage des préparations microscopiques. La Cellule, 23: 428-433.

Lee, A. B. 1913.—The Microtomist's Vade-mecum. 7th. Edition.